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DEPARTMENT OF THE INTERIOR.

BUREAU OF GOVERNMENT LABORATORIES.

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REPORT ON BACILLUS VIOLACEOUS MANILÆ:
A PATHOGENIC MICROORGANISM.

By PAUL G. WOOLLEY, M. D.

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DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
OFFICE OF THE SUPERINTENDENT OF LABORATORIES,

Manila, P. I., May 13, 1904.

SIR: I have the honor to transmit herewith a "Report on Bacillus Violaceus Manilæ, a Pathogenic Microörganism," by Dr. Paul G. Woolley, Assistant Director of the Serum Laboratory.

I am, very respectfully,

PAUL C. FREER,
Superintendent Government Laboratories.

Hon. DEAN C. WORCESTER,
Secretary of the Interior, Manila, P. I.

DEPARTMENT OF THE INTERIOR,
BUREAU OF GOVERNMENT LABORATORIES,
BIOLOGICAL LABORATORY,

Manila, P. I., May 12, 1904.

SIR: I have the honor to submit herewith and recommend for publication a "Report on Bacillus Violaceus Manilæ, a Pathogenic Microörganism," by Dr. Paul G. Woolley, at present Assistant Director of the Serum Laboratory.

Very respectfully,

RICHARD P. STRONG,
Director Biological Laboratory.

Dr. PAUL C. FREER,
Superintendent Bureau of Government Laboratories,
Manila, P. I.

REPORT ON *BACILLUS VIOLACEUS* MANILÆ: A PATHOGENIC MICROÖRGANISM.

By PAUL G. WOOLLEY, M. D.,
Assistant Director Serum Laboratory.

I can find no account in literature of a pathogenic organism similar to the one of which a description follows, but there are one or two bacilli which resemble it so closely in morphologic and cultural characteristics that it seems wisest to consider the present germ as simply a variety of these well known but at least usually nonpathogenic forms.

The group to which reference is made is the one, the individual members of which are discussed collectively by Migula (*System der Bakterien*, 1900, II, 939) under the title of *Pseudomonas violacea*, and which are separately described by other authors as *B. violaceus*, *B. violaceus berolinensis*, *B. violaceus lutetiensis*, *B. violaceus laurentius*, etc.

The organisms are described by these authors as bacilli, which are short or of medium length, sometimes somewhat bent, and with rounded ends. The measurements are about 1 by 0.65 μ , although some individuals may attain a length of 3 μ . In cultures on agar and potato, formations much resembling spores, but which are like these reproductive bodies in appearance only, are seen within the individual rods. These pseudospores are considered to be either plasma globules or vacuoles, and true spores have not been demonstrated. The rods are motile—sometimes, as in cultures kept at body temperature, quite actively so. They possess a single polar flagellum. Deep colonies in gelatin have an irregular, rounded appearance, with projecting filaments. On the surface the colonies form small depressions, in which blue-gray masses of bacilli are seen. The color gradually becomes more intense, and the straggling filaments grow out into the surrounding medium. On agar the growth is blackish violet. Blood serum is liquefied—peptonized. On potato the growth spreads over the surface of the

medium and forms a rich pigment, which does not stain the whole fragment of the vegetable but only that portion immediately beneath the growth. Broth is clouded, and a dirty white sediment falls to the bottom of the tube and takes on a pale violet color. No pellicle is formed on the surface. The organisms grow fast at room temperature, but more slowly than most water bacteria, and they do not grow beneath the isinglass. They are most readily stained with hot carbol-fuchsin, and are decolorized by Gram's method.

B. violaceus manilæ corresponds with the above description in some details, but in some it does not. The organism is a short rod, measuring approximately 0.5 by 1 to 1.5 μ . Occasional rods are even longer. It stains with the usual aniline dyes, best perhaps with carbol-thionin, or with carbol-fuchsin or gentian-violet diluted five times with water. It is not stained by Gram's method, and is not "acid fast." When well stained by any of the above-mentioned solutions, it appears as a short, thin bacillus, very frequently slightly bent, and with rounded ends. It does not, as a rule, stain uniformly, and may show one or more clear spaces which are not tinted and which appear remarkably like spores. These clear spaces can not be stained by the usual methods used in coloring spores. The organisms are generally single, sometimes in pairs, occasionally arranged in short chains of three or four individuals. They are motile, usually sluggishly turning and twisting, but frequently single rods may be seen to cross the field of the microscope with a more rapid, wriggling motion. Each organism possesses one polar flagellum. In rare instances two flagella may be distinguished springing from the same pole of the bacillus.

On agar plates, within twenty-four hours at 37° C., colonies appear as small, round, violet-gray spots. These slowly enlarge and become deeper in color. The maximum depth of color is attained in from forty-eight to seventy-two hours. As the colonies grow, their margins become slightly irregular and with a rather indefinite concentric arrangement of layers, which are somewhat thicker toward their peripheries, with the result that the centers of the colonies have a less intense blue color than the edges. These masses of organisms are slightly tenacious, but after removal from the medium are readily dissociated in water. The growth on agar slants is similar to that on plates, extension being slow but rather even, resulting in a very slight crenation. The water of conden-

sation is clouded and bluish, with a blue sediment. In stab cultures in agar the growth is scant, and no pigment is formed below the surface.

Gelatin is liquefied slowly at the temperature of the ice box, the growth forming a funnel-shaped area with a cup-shaped upper portion. The sediment is bluish.

Bouillon is diffusely clouded. A delicate pellicle is formed, which, upon 1 per cent alkaline (to phenolphthaline) material, is but slightly pigmented, but which is better developed and bluer on a 1 per cent acid liquid. The sediment in 1 per cent acid bouillon is pale violet blue and rather viscid.

Dunham's peptone solution is also clouded, and a thin, pigmented pellicle is produced. A sediment is deposited in this medium which resembles that in broth but in which more pigment is present. The whole medium becomes diffusely bluish within forty-eight hours.

On potato, the growth extends over the whole surface of the medium and to the water of condensation. The pigment production is luxuriant. The superficial layers of all the solid media are stained by the soluble blue pigment. In sugar containing media—glucose and lactose—no gas is formed.

The reaction of milk, after seven days, is slightly acid, but there is no coagulation. However, the casein is peptonized, and the upper third of the tube becomes almost clear and faintly violet.

The organism thrives better and produces pigment somewhat more freely at 37° C., than at lower temperatures. It does not grow at all well and does not produce any pigment below the surface of a solid medium. It is almost an obligatory aërob. It is killed by boiling for one minute, by a temperature of 67° C. after an exposure of five minutes, and by a temperature of 57° C. after one hour. It does not form spores.

The pigment is soluble in alcohol, giving a deep, rich, violet solution. It is slightly soluble in water, barely dissolved by ether, and insoluble in chloroform.

The organism described above has been isolated from three carbabs, which died suddenly with such noticeable absence of clinical symptoms that acute hemorrhagic septicæmia was suspected. In two of these cases Dr. Jobling obtained cultures of this organism from the lymph glands and lungs, and in the third case the writer found it predominating in cultures from the same organs. Later on, in the press of work, the cultures from the first cases were lost,

and only those from the third were used in the following investigation. However, Dr. Jobling had injected his organism into guinea pigs, producing the same kind of lesions which I shall describe. The details of the first autopsies are meager. Little can be said, except that the prescapular glands resembled those to be described in connection with the third case.

Like the first ones, the third animal died suddenly. It had had no rise in temperature, as far as the records of the corral show, and symptoms of rinderpest were absent. On removing the hide none of the gross lesions of hemorrhagic septicæmia or surra were encountered, neither hemorrhages nor oedemas. The prescapular glands were enlarged and intensely injected, but neither showed true hemorrhages nor necrotic areas. There were a few small petecchiæ under the visceral pericardium and under the endocardium of the left ventricle. The lungs were not collapsed, but were for the most part crepitant. The surfaces of these organs were dotted with small, pale areas varying in size from that of a pinhead to a small hazelnut. These areas were firm and projected slightly above the surface of the pleura. They were not round, but irregularly stellate, and each was surrounded by an appreciable zone of congestion. On section they appeared granular and gray, and with no indication of caseation or suppuration. Other noncrepitant areas which occurred chiefly along the anterior margins of the lungs had much the appearance of red infarcts. They were dark red and raised above the general surface of the organ in which they occurred. On section they were dark and moist, simulating the stage of red hepatization in pneumonia. The lungs on section were generally pale and oedematous, and in their substance contained large numbers of the small miliary nodules similar to those seen upon their surface. The spleen appeared to be normal. The liver showed no macroscopic changes. The gall bladder was somewhat larger than normal, but the stomach showed no pathologic lesions visible to the naked eye, nor were any found in the trachea, pharynx, or tonsils. The kidneys were perhaps a trifle pale. The intestinal tract was normal.

Smears from the prescapular glands showed a few very small organisms, which appeared as diplococci or polar-stained bacilli, and a large number of somewhat larger rods; some of these irregularly stained, and others curved. Cover-glass preparations made

from the nodules of the lungs showed almost nothing but small bacilli, which stained unevenly. Smears from the heart showed no organisms.

Cultures from the blood gave no growth after some days, and those from the lung lesions produced a few colonies of a large thick bacillus, resembling *B. subtilis*, and other colonies in very much larger numbers (in one plate, these latter only), which developed a blue color after twenty-four hours at 37° C. and which were composed of the organisms which have been described. In cultures from the prescapular glands two organisms were demonstrated. One was identical with the chromogenic one isolated from the lungs; the other, a small coccus, arranged mostly in clumps, and which, after several days, produced a very faint yellow color. Both of these organisms—the coccus and the chromogenic bacillus—were studied carefully. One was in all probability a modified *Staphylococcus aureus*, which produced a minimum of pigment, coagulated milk very slowly with coincident reduction of the litmus, and which did not kill guinea pigs in reasonable doses, either when injected into the peritoneal cavity, or under the skin. The other has been described in its cultural and morphological character, and corresponds quite closely with *Bacillus violaceus* (Schröter), or *Pseudomonas violacea* (Migula). In none of the books to which we have access have I been able to find any reference to the pathogenicity of *Bacillus violaceus*. This makes the following experimental study of some interest:

One cubic centimeter of a forty-eight-hour-old culture from the lung of the carabao (No. 431) was injected under the skin of a guinea pig (No. 329). The animal became quite ill within the next twenty-four hours and a large semifluctuating mass appeared at the site of the inoculation. Coincidentally the temperature, which previously had been running between 36.5° and 38.4° C., rose to 39.8° C. and then dropped gradually until the animal died. Death occurred on the fifth day after infection.

At autopsy a large area of necrosis was found under the skin about the point of inoculation. This was surrounded by tissue in a state of coagulation necrosis, in which were occasional lacunæ filled with a dark, gelatinous material. There was no true pus and no hemorrhages appeared, although the adjacent tissues showed extensive congestion. The peritoneal cavity contained a small amount of a clear fluid. The lungs were the seat of large and small

hemorrhagic infarcts, the lower lobes of both sides being almost completely infarcted. The spleen was markedly enlarged, was very dark, soft, and mottled with miliary gray spots, resembling focal necroses. The liver was also thickly studded with similar areas. There were no macroscopic lesions in the heart or kidneys, though the latter were pale. The adrenals were markedly enlarged, their medullæ congested, and with small areas of necrosis in the cortices. The lymph glands of the axillæ and groins were enlarged and injected. Nothing abnormal was remarked in the intestines, stomach, or bladder.

Smears were made from the subcutaneous tissues at the site of the primary lesions from the lungs, spleen, liver, lymph glands, and heart. All save those from the heart showed numbers of rods morphologically identical with those which had been injected. The organism was recovered in pure cultures from the liver, peritoneal cavity, lungs, the primary lesion, and the heart.

A second guinea pig (No. 458) was inoculated intraperitoneally with one-half of a cubic centimeter of a bouillon culture obtained from the carabao. It survived the operation.

From the cultures obtained from the first guinea pig a second (No. 327) was inoculated with one-half cubic centimeter of an emulsion made with three loopfuls of a twenty-four-hour-old agar culture in 5 cubic centimeters of a normal salt solution. The animal died on the third day after inoculation. The anatomical picture was the same as that in the first guinea pig, except that in the lungs small miliary necroses were present in place of the infarcts noticed before. Pure cultures of the experimental organisms were obtained from the heart, liver, and the subcutaneous lesions.

With 1 cubic centimeter of an emulsion made with three loopfuls of the organisms obtained from this last animal and 5 cubic centimeters of salt solution, a rabbit (No. 474) was inoculated subdermally. It died within thirty-six hours. Autopsy showed a bloody fluid in the peritoneal cavity, a widespread hemorrhagic lesion at the site of inoculation with no suppuration, but with necroses in the liver. The organisms were recovered in pure culture from the peritoneal cavity, liver, and heart. At the same time a guinea pig, inoculated with an equal amount of the same material, died within twenty hours. The only macroscopic lesions were miliary abscesses of the liver. Cultures were obtained from the heart, liver, and peritoneal cavity.

A cat (No. 366) was inoculated under the skin of the belly with 1 cubic centimeter of an agar suspension of a culture obtained from guinea pig No. 327. The succeeding day a large semifluctuant mass surrounded the point of inoculation. The second day after infection this abscess was discharging externally by a sinus, the edges of which were ragged and about which the skin was semi-necrotic. Eventually all the skin about this first sinus sloughed, leaving an ulcer measuring 5 by 5 centimeters, whose base was on the subjacent muscles and whose edges were regular, indurated, and undermined. This ulcer gradually healed, and the animal showed a complete skin on the thirtieth day after inoculation. When recovery had been established, a second dose of a suspension of the organisms from No. 474 was introduced hypodermically. No lesion was produced. The serum from this animal taken at this time agglutinated the specific organisms in a dilution of 1 to 60 after about forty minutes, in dilution of 1 to 200 after about one hour and fifteen minutes.

Five rabbits were inoculated with different amounts of the organisms to show, if possible, what variations occurred in the lesions. The first (No. 454) received one-half loopful; the second (No. 455), one; the third (No. 436), two; the fourth (No. 435), three, and the fifth (No. 433), four, of a culture obtained from rabbit No. 474. All of these animals died—Nos. 454, 455, and 436 in three days, No. 435 in four days, and No. 433 in five days after inoculation. From all, the organism was recovered from the tissues and heart's blood, except in two cases (No. 435 and 436), in which the cultures from the heart were negative.

The progressive changes in the organs of these animals were noticeable and interesting. In animal No. 454 the subcutaneous jelly-like œdema was present; there were miliary abscesses in the liver, none in the spleen, none in the lungs, which were simply injected. In animal No. 455 there were a few nodules in the lungs and in the liver; the other organs appeared like those in animal No. 454. In animal No. 436 there were large abscesses in the lungs, and but a few small ones in the liver. Animal No. 435 showed similar lesions. In No. 433 there were a few very small abscesses in the liver and spleen, while the lungs were generally consolidated, showing comparatively large areas composed of collections of miliary abscesses. In other words, the lesions varied as the dose of the organisms, and while with small doses the liver was

more prone to be affected, with large ones the lungs were more prominently diseased. This may or may not be true for other animal species. At any rate, the principal feature of the pathogenic action of the bacillus is its necrotizing power.

A dog (No. 515) was inoculated subcutaneously with 1 cubic centimeter of an agar suspension of a culture from No. 474. Several days later a local lesion appeared, which resembled that produced in the cat, but which was less extensive and healed more rapidly. About three weeks afterwards this dog received 1 cubic centimeter of similar material in the femoral vein. Following this injection the animal was irritable, eating but little for a few days. On the third day, and continuing for four days, tremors were noticed in the head and limbs, the animal lying quietly without seeming to suffer. He subsequently became entirely well.

In the case of another dog (No. 516), in which the organisms were introduced into the trachea, no illness followed.

A calf was inoculated in the dewlap with 2 cubic centimeters of the same material which was used with the dogs. The day following inoculation there was a large, painful, oedematous mass visible in the region of infection. This enlarged until it reached the size of a large fist, and then gradually disappeared.

Inoculation into the peritoneal cavity of 1 cubic centimeter of a forty-eight-hour-old agar culture suspended in salt solution caused the death of a rabbit (No. 492) within twelve hours. At autopsy there was nothing to be seen except evidence of an acute peritonitis. Cultures from heart and peritoneal cavity were positive and pure.

Intravenous inoculation of one-third of a cubic centimeter of a forty-eight-hour-old agar suspension killed a rabbit (No. 490) within ten hours. No lesions were visible to the naked eye. Cultures made from the heart, peritoneal cavity, and pleura showed the organisms in uncontaminated growths.

Feeding experiments were negative. Large quantities of virulent agar cultures were fed to two monkeys (Nos. 488 and 489) with no ill effects.

Experiments upon serum reactions were begun with a monkey (No. 468), which had received a dose of 1 cubic centimeter of an emulsion made from a twenty-four-hour-old agar culture of the organisms isolated from the carabao. The monkey did not succumb to this first subcutaneous injection, and four days later a second one was made with $1\frac{1}{2}$ cubic centimeters of an emulsion

made with a twenty-four hour culture of the organisms isolated from the second guinea pig (No. 327). The day following this second injection a small quantity of blood was withdrawn and its agglutinative powers tested. In dilutions of 1 to 20 a complete agglutination was present at the end of fifteen minutes. In 1 to 40 a complete reaction was given in half an hour. In a dilution of 1 to 200 the result was positive and complete in one hour.

Later this animal was inoculated subcutaneously with $1\frac{1}{2}$ cubic centimeters of an agar suspension made with a culture of the organisms recovered from animal No. 474. Two days afterwards a large slough appeared on the abdomen about the point of inoculation, and the animal was very ill. It was killed and the blood drained off into a sterile tube. The serum obtained from this blood was tested for its agglutinative and bactericidal powers. In a dilution of 1 to 200 agglutination was complete in twenty minutes, 1 to 400 in thirty minutes, 1 to 600 in forty-five minutes, 1 to 1,000 in fifty minutes, 1 to 2,000 incomplete after one hour, 1 to 4,000 was negative.

This experiment was repeated in small tubes, so that the reaction could be watched with the naked eyes. The tubes were allowed to stand overnight. The next day all the organisms were contained in a precipitate, except in the control tube in which the liquid was still cloudy. Cultures made from all these tubes gave luxuriant growths. No appreciable bactericidal action was present.

To determine whether or not a soluble toxin was produced, a four-day-old culture in peptone was filtered through a Pasteur-Chamberland bougie F. The filtrate was kept at 37° C. overnight and no growth occurred. The next day 5 cubic centimeters of the material was injected under the skin of a monkey (No. 509). Following this there was no sign of toxæmia, no rise of temperature, nor any sign of altered health in the animal.

After it had been proved that even very small amounts of the living organisms would cause death of small animals and at the same time produce the specific lesions without the appearance of any appreciable immunity, cultures were heated to 57° C. for one hour, and these dead cultures, suspended in normal salt solution, were used for injection.

This material was used subcutaneously in a guinea pig (No. 486) and a monkey (No. 411). In the case of the monkey one cubic centimeter of an agar suspension was used for the first injection.

There was no appearance of a reaction until the fifth day, when the temperature rose one degree above the normal one for the animal. The day following this reaction a second injection of the same amount of the same material was given. On the fourth day following, the blood was tested for its agglutinating powers. Complete agglutination was accomplished in dilutions of 1 to 400 in fifteen minutes. A third inoculation was then made with 0.75 cubic centimeter (intraperitoneal) and 0.75 cubic centimeter (subcutaneous) of the same material which had been used for the other inoculations, and four days later a fourth inoculation was made with $2\frac{1}{2}$ cubic centimeters subcutaneously. Two days after the last inoculation the monkey was found dead. At autopsy there was a small pocket of a bluish material at the site of the last inoculation. Cultures from this lesion, from the heart, liver, and spleen were entirely negative, and the media was perfectly sterile after five days. The serum taken from the heart at autopsy gave a perfect agglutination in a dilution of 1 to 1,000 in forty minutes. The guinea pig suffered no harm from the inoculations.

Later, using cultures of the organisms from animal No. 474, and which had been killed by heating to 70° C. for forty-six minutes, a dog was inoculated with a series of injections. He remained well throughout the time. However, after the third inoculation his blood agglutinated in a dilution of 1 to 20 only, after one hour.

The essential lesions produced in experimental animals are found at the site of inoculation, in the lungs, liver, lymph glands, and spleen.

At the site of inoculation, when infection has been caused subcutaneously, there is a wide area of necrosis with local and circumambient œdema resembling the lesions produced by the diphtheria bacillus. The whole area may undergo necrosis, become gangrenous, and slough away.

In the rabbits the œdema has been more marked than the necrosis. In the cat the necrosis occurred with but little œdema. The monkeys showed œdema as well as necrotic and gangrenous processes, as did the guinea pigs.

The lesions in the parenchymatous organs are miliary abscesses, which may show a suppurative stage. In the lungs and liver the surface of these abscesses may be covered with a fibrinous exudate. The bacilli are found in all the lesions.

The losses in stock due to infection with *B. violaceus manilæ* are

not extensive, and there seems to be little danger of an epidemic. In all our work we have seen but these three cases. Each was in a different herd, and these herds were widely separated from each other.

So far as treatment is concerned there is little to be said. All the animals have died so suddenly and unexpectedly that there was no time either for experiment or speculation on this subject.

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